AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

Claim 1. (Currently Amended) A diagnostic or prognostic assay for prostate cancer ~~or liver cancer ina~~ in a subject, said cancer characterized by abnormal methylation of cytosine at at least one CpG site in a target region within the glutathione S-transferase (GST) Pi gene and/or it regulatory flanking sequences, wherein said assay comprises the steps of:

(i) isolating DNA from a test subject,

(ii) treating the isolated DNA such that unmethylated cytosines are converted to uracil or another nucleotide capable of forming a base pair with adenine while methylated cytosines are unchanged or converted to a nucleotide capable of forming a base pair with guanine;

(~~ii~~iii) carrying out amplification of said treated ~~isolated~~ DNA so as to amplify a target region of the GST-Pi gene and/or its regulatory flanking sequences which includes a site or sites at which abnormal cytosine methylation characteristic of the cancer occurs, the amplification being selective in that it only amplifies the target region if the said site or sites at which abnormal cytosine methylation occurs is/are methylated, and

(~~iii~~iv) detecting the presence of amplified DNA, wherein the detection of amplified DNA is indicative of methylation, and thereby indicative of said cancer,

wherein the amplifying step (iii) is used to amplify a target region, wherein said target region is within the GST-Pi gene and/or its regulatory flanking sequences ~~defined by (and inclusive of) sites 342-343 of SEQ ID NO: 52 to CpG sites 581-582 of SEQ ID NO: 54~~ and said target region comprises nucleotides 836-1117 (CpG sites -43 to -14) of SEQ ID NO:60~~,~~

~~wherein the isolated DNA is not treated with a methylation sensitive restriction endonuclease prior to amplification in step (ii)~~.

Claim 2. (Cancelled).

Claim 3. (Previously Presented) An assay according to claim 1, wherein the amplifying step involves polymerase chain reaction (PCR) amplification.

Claim 4. (Currently Amended) An assay according to claim 1~~2~~, wherein said amplification step comprises PCR amplification utilizing a reverse primer including guanine at at least one site whereby, upon the reverse primer annealing to the treated DNA, said guanine will either form a base pair with a methylated cytosine (or another nucleotide to which the methylated cytosine has been converted through said treatment) if present, or will form a mismatch with uracil (or another nucleotide to which unmethylated cytosine has been converted through said treatment).

Claim 5. (Previously Presented) An assay according to claim 4, wherein said PCR amplification utilizes a forward primer including cytosine at least one site(s) corresponding to cytosine nucleotides that are abnormally methylated in the DNA of a subject with the cancer being assayed.

Claim 6. (Original) An assay according to Claim 5, wherein the primers are of 12 to 30 nucleotides in length.

Claim 7. (Previously Presented) An assay according to claim 6, wherein the primers are selected so as to anneal to a sequence within the target region that includes two to four cytosine nucleotides that are abnormally methylated in the isolated DNA of a subject with the cancer being assayed.

Claim 8. (Currently Amended) An assay according to claim 1~~2~~, wherein the treatment of the isolated DNA involves reacting the isolated DNA with bisulphite.

Claim 9. (Original) An assay according to claim 8, wherein the amplifying step involves polymerase chain reaction (PCR) amplification.

Claim 10. (Original) An assay according to claim 9, wherein said PCR amplification utilizes a reverse primer including guanine at at least one site whereby, upon the reverse primer annealing to the treated DNA, said guanine will either form a base pair with a methylated cytosine if present, or will form a mismatch with uracil.

Claim 11. (Previously Presented) An assay according to claim 10, wherein said PCR amplification utilizes a forward primer including cytosine at at least one site(s) corresponding to cytosine nucleotides that are abnormally methylated in the isolated DNA of a subject with the cancer being assayed.

Claim 12. (Original) An assay according to claim 11, wherein the primers are of 12 to 30 nucleotides in length.

Claim 13. (Previously Presented) An assay according to claim 12, wherein the primers are selected so as to anneal to a

sequence within the target region that includes two to four cytosine nucleotides that are abnormally methylated in the DNA of a subject with cancer being assayed.

Claim 14. (Previously Presented) An assay according to Claim 1, wherein said DNA is isolated from cells from tissue, blood, blood serum, blood plasma, semen, urine, lymph, or bone marrow.

Claims 15-18. (Cancelled).

Claim 19. (Currently Amended) An assay according to claim ~~17~~1, wherein the amplifying step (iii) is used to amplify a target region ~~within the region of the GST Pi gene and its regulatory flanking sequences defined by (and inclusive of)~~comprising nucleotides 836-1155 (CpG sites -43 to ~~+10~~-8) of SEQ ID NO:60.

Claim 20. (Currently Amended) An assay according to claim ~~17~~1, wherein the amplifying step (iii) is used to amplify a target region ~~within the region of the GST Pi gene and its regulatory flanking sequences defined by (and inclusive of)~~comprising nucleotides 836-1279 (CpG sites -43 to ~~-14~~+10) of SEQ ID NO:60.

Claim 21. (Currently Amended) An assay according to claim ~~17~~1, wherein the amplifying step (iii) is used to amplify a target region ~~within the region of the GST Pi gene and its regulatory flanking sequences defined by (and inclusive of)~~comprising nucleotides 836-1941 (CpG sites -43 to ~~-8~~+55) of SEQ ID NO:60.

Claim 22. (Cancelled).

Claim 23. (Currently Amended) An assay according to claim 5, wherein if either or both of the reverse or forward primers anneal to a sequence within the target region that includes any or all of the CpG sites at nucleotides 925, 943, 1063, 1080, 1084, 1116 (CpG sites -36, -32, -23, -20, -19~~and~~, -14, respectively), then said PCR amplification further utilizes equivalent reverse and/or forward primers including a redundant nucleotide(s) at the position(s) within their sequence(s) corresponding to cytosine or methylated cytosine of CpG sites -36, -32, -23, -20, -19, ~~and~~ -14, respectively.

Claims 24-25. (Cancelled).

Claim 26. (Currently Amended) An assay according to claim ~~17~~1, wherein the amplification involves PCR amplification using primer pairs consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers

CGCGAGGTTTTCGTTGGAGTTTCGTCGTC (SEQ ID NO: 1)

CGTTATTAGTGAGTACGCGCGGTTC (SEQ ID NO: 2)

YGGTTTTAGGGAATTTTTTTTCGC (SEQ ID NO: 3)

YGGYGYGTTAGTTYGTTGYGTATATTTC (SEQ ID NO: 4)

GGGAATTTTTTTTCGCGATGTTTYGGCGC (SEQ ID NO: 5)

TTTTTAGGGGGTTYGGAGCGTTTC (SEQ ID NO: 6)

GGTAGGTTGYGTTTATCGC (SEQ ID NO: 7)

Reverse Primers

TCCCATCCCTCCCCGAAACGCTCCG (SEQ ID NO: 8)

GAAACGCTCCGAACCCCCTAAAAACCGCTAACG (SEQ ID NO: 9)

CRCCCTAAAATCCCCRAAATCRCCGCG (SEQ ID NO: 10)

ACCCCRACRACCRCTACACCCCRAACGTCG (SEQ ID NO: 11)

CTCTTCTAAAAAATCCCRCRAACTCCCGCCG (SEQ ID NO: 12)

AAAACRCCCTAAAATCCCCGAAATCGCCG (SEQ ID NO: 13)

AACTCCCRCCGACCCCAACCCCGACGACCG (SEQ ID NO: 14)

AAAAATTCRAATCTCTCCGAATAAACG (SEQ ID NO: 15)

AAAAACCRAAATAAAAACCACACGACG (SEQ ID NO: 16),

wherein Y is C, T or a mixture thereof, and R is A, G or a mixture thereof.

Claim 27. (Currently Amended) An assay according to claim ~~17~~1, wherein the amplification step involves PCR amplification using primer pairs consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers

CGCGAGGTTTTCGTTGGAGTTTCGTCGTC (SEQ ID NO: 1)

CGTTATTAGTGAGTACGCGCGGTTC (SEQ ID NO: 2)

Reverse Primers

TCCCATCCCTCCCCGAAACGCTCCG (SEQ ID NO: 8)

GAAACGCTCCGAACCCCCTAAAAACCGCTAACG (SEQ ID NO: 9).

Claim 28. (Currently Amended) An assay according to claim ~~17~~1, wherein the amplification step involves PCR amplification using primer pairs consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers

YGGTTTTAGGGAATTTTTTTTCGC (SEQ ID NO: 3)

YGGYGYGTTAGTTYGTTGYGTATATTTC (SEQ ID NO: 4)

GGGAATTTTTTTTCGCGATGTTTYGGCGC (SEQ ID NO: 5)

Reverse Primers

CRCCCTAAAATCCCCRAAATCRCCGCG (SEQ ID NO: 10)

ACCCCRACRACCRCTACACCCCRAACGTCG (SEQ ID NO: 11)

CTCTTCTAAAAAATCCCRCRAACTCCCGCCG (SEQ ID NO: 12)

AAAACRCCCTAAAATCCCCGAAATCGCCG (SEQ ID NO: 13)

AACTCCCRCCGACCCCAACCCCGACGACCG (SEQ ID NO: 14),

wherein Y is C, T or a mixture thereof and R is A, G or a mixture thereof.

Claim 29. (Currently Amended) An assay according to claim ~~17~~1, wherein the amplification step involves PCR amplification using primer pairs consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers

TTTTTAGGGGGTTYGGAGCGTTTC (SEQ ID NO: 6)

GGTAGGTTGYGTTTATCGC (SEQ ID NO: 7)

Reverse Primers

AAAAATTCRAATCTCTCCGAATAAACG (SEQ ID NO: 15)

AAAAACCRAAATAAAAACCACACGACG (SEQ ID NO: 16),

wherein Y is C, T or a mixture thereof, and R is A, G or a mixture thereof.

Claims 30-48. (Cancelled).

Claim 49. (Withdrawn) A primer or probe comprising a nucleotide sequence selected from the group consisting of:

CGCGAGGTTTTCGTTGGAGTTTCGTCGTC (SEQ ID NO: 1)

CGTTATTAGTGAGTACGCGCGGTTC (SEQ ID NO: 2)

YGGTTTTAGGGAATTTTTTTTCGC (SEQ ID NO: 3)

YGGYGYGTTAGTTYGTTGYGTATATTTC (SEQ ID NO: 4)

GGGAATTTTTTTTCGCGATGTTTYGGCGC (SEQ ID NO: 5)

TTTTTAGGGGGTTYGGAGCGTTTC (SEQ ID NO: 6)

GGTAGGTTGYGTTTATCGC (SEQ ID NO: 7)

AAAAATTCRAATCTCTCCGAATAAACG (SEQ ID NO: 8)

AAAAACCRAAATAAAAACCACACGACG (SEQ ID NO: 9)

TCCCATCCCTCCCCGAAACGCTCCG (SEQ ID NO: 10)

GAAACGCTCCGAACCCCCTAAAAACCGCTAACG (SEQ ID NO: 11)

CRCCCTAAAATCCCCRAAATCRCCGCG (SEQ ID NO: 12)

ACCCCRACRACCRCTACACCCCRAACGTCG (SEQ ID NO: 13)

CTCTTCTAAAAAATCCCRCRAACTCCCGCCG (SEQ ID NO: 14)

AAAACRCCCTAAAATCCCCGAAATCGCCG (SEQ ID NO: 15)

AACTCCCRCCGACCCCAACCCCGACGACCG, (SEQ ID NO: 16),

wherein Y is a mixture of C and T, and R is a mixture of A and G.

Claim 50. (Withdrawn) A probe comprising a nucleotide sequence selected from the group consisting of:

AAACCTAAAAAATAAACAAACAA (SEQ ID NO: 17)

GGGCCTAGGGAGTAAACAGACAG (SEQ ID NO: 18)

CCTTTCCCTCTTTCCCARRTCCCCA (SEQ ID NO: 19)

TTTGGTATTTTTTTTTCGGGTTTTAG (SEQ ID NO: 20)

CTTGGCATCCTCCCCCGGGCTCCAG (SEQ ID NO: 21)

GGYAGGGAAGGGAGGYAGGGGYTGGG (SEQ ID NO: 22).

Claims 51-76. (Cancelled).

Claim 77. (New) A diagnostic or prognostic assay for prostate cancer in a subject, said cancer characterized by abnormal methylation of cytosine at at least one CpG site in a

target region within the glutathione S-transferase (GST) Pi gene, wherein said assay comprises the steps of:

(i) isolating DNA from a test subject,

(ii) carrying out amplification of said isolated DNA so as to amplify a target region of the GST-Pi gene and/or its regulatory flanking sequences which includes a site or sites at which abnormal cytosine methylation characteristic of the cancer occurs, the amplification being selective in that it only amplifies the target region if the said site or sites at which abnormal cytosine methylation occurs is/are methylated, and

(iii) detecting the presence of amplified DNA, wherein the detection of amplified DNA is indicative of methylation, and thereby indicative of said cancer,

wherein the amplifying step (ii) is used to amplify a target region comprising nucleotides 1232-1941 (CpG sites +1 to +55) of SEQ ID NO:60.

Claim 78. (New) An assay according to claim 77, wherein prior to the amplifying step, the isolated DNA is treated such that unmethylated cytosines are converted to uracil or another nucleotide capable of forming a base pair with adenine while methylated cytosines are unchanged or converted to a nucleotide capable of forming a base pair with guanine.

Claim 79. (New) An assay according to claim 77, wherein the amplifying step involves polymerase chain reaction (PCR) amplification.

Claim 80. (New) An assay according to claim 78, wherein said amplification step comprises PCR amplification utilizing a reverse primer including guanine at at least one site whereby,

upon the reverse primer annealing to the treated DNA, said guanine will either form a base pair with a methylated cytosine (or another nucleotide to which the methylated cytosine has been converted through said treatment) if present, or will form a mismatch with uracil (or another nucleotide to which unmethylated cytosine has been converted through said treatment).

Claim 81. (New) An assay according to claim 80, wherein said PCR amplification utilizes a forward primer including cytosine at least one site(s) corresponding to cytosine nucleotides that are abnormally methylated in the DNA of a subject with the cancer being assayed.

Claim 82. (New) An assay according to claim 81, wherein the primers are of 12 to 30 nucleotides in length.

Claim 83. (New) An assay according to claim 82, wherein the primers are selected so as to anneal to a sequence within the target region that includes two to four cytosine nucleotides that are abnormally methylated in the isolated DNA of a subject with the cancer being assayed.

Claim 84. (New) An assay according to claim 78, wherein the treatment of the isolated DNA involves reacting the isolated DNA with bisulphite.

Claim 85. (New) An assay according to claim 84, wherein the amplifying step involves polymerase chain reaction (PCR) amplification.

Claim 86. (New) An assay according to claim 85, wherein said PCR amplification utilizes a reverse primer including guanine at at least one site whereby, upon the reverse primer

annealing to the treated DNA, said guanine will either form a base pair with a methylated cytosine if present, or will form a mismatch with uracil.

Claim 87. (New) An assay according to claim 86, wherein said PCR amplification utilizes a forward primer including cytosine at at least one site(s) corresponding to cytosine nucleotides that are abnormally methylated in the isolated DNA of a subject with the cancer being assayed.

Claim 88. (New) An assay according to claim 87, wherein the primers are of 12 to 30 nucleotides in length.

Claim 89. (New) An assay according to claim 88, wherein the primers are selected so as to anneal to a sequence within the target region that includes two to four cytosine nucleotides that are abnormally methylated in the DNA of a subject with cancer being assayed.

Claim 90. (New) An assay according to claim 77, wherein said DNA is isolated from cells from tissue, blood, blood serum, blood plasma, semen, urine, lymph, or bone marrow.

Claim 91. (New) An assay according to claim 77, wherein the amplifying step is used to amplify a target region comprising nucleotides 1273-1941 (CpG sites +9 to +55) of SEQ ID NO:60.

Claim 92 (New) A diagnostic or prognostic assay for liver cancer in a subject, said cancer characterized by abnormal methylation of cytosine at at least one CpG site in a target region within the glutathione S-transferase (GST) Pi gene and/or

its regulatory flanking sequences, wherein said assay comprises the steps of:

(i) isolating DNA from a test subject,

(ii) carrying out amplification of said isolated DNA so as to amplify a target region of the GST-Pi gene and/or its regulatory flanking sequences which includes a site or sites at which abnormal cytosine methylation characteristic of the cancer occurs, the amplification being selective in that it only amplifies the target region if the said site or sites at which abnormal cytosine methylation occurs is/are methylated, and

(iii) detecting the presence of amplified DNA, wherein the detection of amplified DNA is indicative of methylation, and thereby indicative of said cancer,

wherein the amplifying step (ii) is used to amplify a target region, wherein said target region is within the GST-Pi gene and/or its regulatory flanking sequences and said target region comprises nucleotides 836-1117 (CpG sites -43 to -14) of SEQ ID NO:60.

Claim 93. (New) An assay according to claim 92, wherein prior to the amplifying step, the isolated DNA is treated such that unmethylated cytosines are converted to uracil or another nucleotide capable of forming a base pair with adenine while methylated cytosines are unchanged or converted to a nucleotide capable of forming a base pair with guanine.

Claim 94. (New) An assay according to claim 92, wherein the amplifying step involves polymerase chain reaction (PCR) amplification.

Claim 95. (New) An assay according to claim 93, wherein said amplification step comprises PCR amplification utilizing a reverse primer including guanine at at least one site whereby, upon the reverse primer annealing to the treated DNA, said guanine will either form a base pair with a methylated cytosine (or another nucleotide to which the methylated cytosine has been converted through said treatment) if present, or will form a mismatch with uracil (or another nucleotide to which unmethylated cytosine has been converted through said treatment).

Claim 96. (New) An assay according to claim 95, wherein said PCR amplification utilizes a forward primer including cytosine at least one site(s) corresponding to cytosine nucleotides that are abnormally methylated in the DNA of a subject with the cancer being assayed.

Claim 97. (New) An assay according to claim 96, wherein the primers are of 12 to 30 nucleotides in length.

Claim 98. (New) An assay according to claim 97, wherein the primers are selected so as to anneal to a sequence within the target region that includes two to four cytosine nucleotides that are abnormally methylated in the isolated DNA of a subject with the cancer being assayed.

Claim 99. (New) An assay according to claim 98, wherein the treatment of the isolated DNA involves reacting the isolated DNA with bisulphite.

Claim 100. (New) An assay according to claim 99, wherein the amplifying step involves polymerase chain reaction (PCR) amplification.

Claim 101. (New) An assay according to claim 100, wherein said PCR amplification utilizes a reverse primer including guanine at at least one site whereby, upon the reverse primer annealing to the treated DNA, said guanine will either form a base pair with a methylated cytosine if present, or will form a mismatch with uracil.

Claim 102. (New) An assay according to claim 101, wherein said PCR amplification utilizes a forward primer including cytosine at at least one site(s) corresponding to cytosine nucleotides that are abnormally methylated in the isolated DNA of a subject with the cancer being assayed.

Claim 103. (New) An assay according to claim 102, wherein the primers are of 12 to 30 nucleotides in length.

Claim 104. (New) An assay according to claim 103, wherein the primers are selected so as to anneal to a sequence within the target region that includes two to four cytosine nucleotides that are abnormally methylated in the DNA of a subject with cancer being assayed.

Claim 105. (New) An assay according to claim 92, wherein said DNA is isolated from cells from tissue, blood, blood serum, blood plasma, semen, urine, lymph, or bone marrow.

Claim 106. (New) An assay according to claim 92, wherein the amplification step (ii) is used to amplify a target region comprising nucleotides 836-1941 (CpG sites -43 to +55) of SEQ ID NO:60.

Claim 107. (New) An assay according to claim 106, wherein the target region excludes any or all of the CpG sites at

nucleotides 925, 943, 1063, 1080, 1084, 1116 (CpG sites -36, -32, -23, -20, -19, -14, respectively) of SEQ ID NO:60.

Claim 108. (New) An assay according to claim 95, wherein if either or both of the reverse or forward primers anneal to a sequence within the target region that includes any or all of the CpG sites at nucleotides CpG sites at nucleotides 925, 943, 1063, 1080, 1084, 1116 (CpG sites -36, -32, -23, -20, -19, -14, respectively), then said PCR amplification further utilizes equivalent reverse and/or forward primers including a redundant nucleotide(s) at the position(s) within their sequence(s) corresponding to cytosine or methylated cytosine of CpG sites -36, -32, -23, -20, -19, -14, respectively.

Claim 109. (New) A diagnostic or prognostic assay for liver cancer in a subject, said cancer characterized by abnormal methylation of cytosine at at least one CpG site in a target region within the glutathione S-transferase (GST) Pi gene and/or its regulatory flanking sequences, wherein said assay comprises the steps of:

(i) isolating DNA from a test subject,

(ii) carrying out amplification of said isolated DNA so as to amplify a target region of the GST-Pi gene and/or its regulatory flanking sequences which includes a site or sites at which abnormal cytosine methylation characteristic of the cancer occurs, the amplification being selective in that it only amplifies the target region if the said site or sites at which abnormal cytosine methylation occurs is/are methylated, and

(iii) detecting the presence of amplified DNA, wherein the detection of amplified DNA is indicative of methylation, and thereby indicative of said cancer,

wherein the amplifying step (ii) is used to amplify a target region within the GST-Pi gene and/or its regulating flanking sequences and said target region comprises nucleotides 1273-1941 (CpG sites +9 to +55) of SEQ ID NO:60.